(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 2 402 034 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
16.04.2014 Bulletin 2014/16

(21) Application number: **11183460.2**

(22) Date of filing: **07.09.2005**

(51) Int Cl.:
A61K 45/00 *(2006.01)* ***A61K 31/787*** *(2006.01)*
A61P 37/04 *(2006.01)* ***A61K 38/16*** *(2006.01)*
A61P 43/00 *(2006.01)* ***A61K 38/40*** *(2006.01)*

(54) **A method for screening for a substance having an action of proliferating natural killer cells**

Verfahren zum Screening einer Substanz, die eine Wirkung auf die Proliferation von NK-Zellen aufweist

Procédé de criblage d'une substance ayant une action de prolifération des cellules tueuses naturelles

(84) Designated Contracting States:
DE FR GB NL

(30) Priority: **15.09.2004 JP 2004268041**

(43) Date of publication of application:
04.01.2012 Bulletin 2012/01

(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
05782272.8 / 1 790 355

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EP 2 402 034 B1

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Description

Field of invention

[0001] The present invention relates to a method for screening for a substance having a natural killer cell proliferating action. The present invention is useful in the fields of medicine, food and so forth.

Background to the Invention

[0002] Natural killer (NK) cells are a group of cells having a cytotoxic activity against various tumor cells, virus infected cells and cells having different major histocompatibility antigens, and their activation and inhibition are regulated primarily by receptors that recognize self and nonself.

[0003] NK cells are considered to have important functions as one type of lymphocytes involved in natural immunity. Against viral infection, in particular, they play a very important role in immune responses in early infection until acquired immunity is established by T lymphocytes and B lymphocytes.

[0004] That is, even if T lymphocytes and B lymphocytes are normal, immunodeficient patients and mice that are deficient in the natural killer function are very easily infected by specific viruses. In recent years, it has been revealed that receptors ofNK cells recognize products of specific viruses.

[0005] Thus, attempts have been made to effectively utilize various functions of NK cells involved in tumor immunity for tumor treatment and removal of virus infected cells that are supposed to become a source of tumors.

[0006] As a method for obtaining NK cells from laboratory animals, a method of collecting cells from the spleen, blood, bone marrow and so forth and purifying them is known. However, since all of these tissues contain NK cells only in low proportions, technical skills and suitable equipment are required to secure NK cells necessary for experiments.

[0007] As for methods for producing or proliferating NK cells, it is well known that if peripheral blood mononuclear cells are cultured in a culture broth to which a large amount of interleukin-2 is added, lymphokine-activated killer lymphocytes (LAK cells) are proliferated within about 1 week in the case of human, and a large amount of NK cells are contained in this culture. Recently, a method has been developed which comprises irradiating cells of the human B cell strain 721.221, in which the major histocompatibility class I (MHC-I) is hardly expressed on the cell surfaces, with a radioactive ray so that the cells should lose division potential, culturing the cells with mononuclear cells in peripheral blood as mixed culture for 5 to 6 days, purifying NK cells from the culture and further continuing culture to obtain a large amount ofNK cells (e.g., Non-patent document 1).

[0008] Further, a method for proliferating human NK cells comprising the step of mixed culture of peripheral blood mononuclear cells and cells of human fibroblast Wilms' tumor line HFWT (Patent document 1), a method for enhancing an activity of animal natural killer lymphocytes utilizing conjugated linoleic acids as an active ingredient (Patent document 2) and so forth have been disclosed. However, the technique disclosed in Patent document 1 is a method for proliferating human NK cells by mixed culture of peripheral blood mononuclear cells and cells of human fibroblast Wilms' tumor line HFWT, and any method for collecting NK cells proliferated *in vivo* and screening for a factor that enhances the natural killer activity are not disclosed in this document. Further, in Patent document 2, changes in killer lymphocytes enhanced by conjugated dienoic linoleic acids in the spleen was followed, and any method for collecting NK cells proliferated *in vivo* and screening for a factor that enhances the natural killer activity are not disclosed in this document.

[0009] At present, the most common method for measuring an activity of NK cells (natural killer activity, NK activity) is a method of observing a cytotoxic activity against human K562 cells or mouse lymphoma cell line Yac-1 by co-culturing a group of cells including Yac-1 cells radiolabeled with ^{51}Cr or the like and measuring radioactivity released in the culture supernatant. Further, a method for measuring the NK activity in living bodies is a method of transplanting tumor cells radiolabeled in the same manner as mentioned above into a living body or infecting an animal with a virus or the like and observing the NK activity, and these methods require a special facility and equipment.

[0010] Lactoferrin is known as a lactoprotein having various actions such as an antibacterial action, immunity activating action and antitumor action. Lactoferrin is a milk-derived glycoprotein that is highly safe and can be continuously taken for a long period of time. Since lactoferrin itself has almost no taste or odor, it is a highly versatile protein as an additive for various food, drugs and feeds.

[0011] Patent document 3 (International Patent Publication in Japanese (Kohyo) No. 2002-515893) proposes a method for stimulating NK cells in a patient which comprises the step of administering a composition containing a modified human lactoferrin to the patient. However, Patent document 3 does not describe the effect of the modified human lactoferrin for actually stimulating NK cells, and its basis has been unclear.

[0012] The Toll-like receptors (TLR) recognize various components of bacteria and are believed to play an important role not only in recognition of bacteria in natural immunity but also in activation of acquired immunity, and also considered to have functions of recognizing various pathogenic components and inducing unique responses. Currently, the Toll-like receptor family in mammals consists of 10 family members, and pathogen-constituting components (ligands) rec-

ognized by each Toll-like receptor have been identified. These ligands, the pathogen-constituting components, include lipids, sugars, proteins, nucleic acids and so forth (Non-patent documents 2 and 3).

[Patent document 1] Japanese Patent Laid-open (Kokai) No. 2001-149069
[Patent document 2] International Patent Publication in Japanese (Kohyo) No. 2001-503430
[Patent document 3] International Patent Publication in Japanese (Kohyo) No. 2002-515893
[Non-patent document 1] Proc. Natl. Acad. Sci., USA, Vol. 94, 1997, pp.13140-13145
[Non-patent document 2] Molecular Medicine, Vol. 39, 2002, pp.238-246
[Non-patent document 3] Molecular Medicine, Vol. 40, 2003, pp.186-193

[0013] WO 02/47612 A. This patent application discloses a number of compositions that are used as dietary supplements. These compositions include colostrum, lactoferrin, β-glucan and citrus pectin.

[0014] Hiroyuki Tsuda et al.: "Lactoferrin niyoru Hatsugan Yobo to Sono Kijo no Kaiseki (Inhibition of Carcinogenesis by Bovine Lactoferrin and Analysis of Mechanisms)", FFI Journal, XX, JP, no.200, 1 January 2002, pages 27-35. This document discloses that bovine lactoferrin has been found to significantly inhibit colon, oesophagus, lung and bladder carcinogenesis in rats when administered orally. Concomitant administration of bovine lactoferrin with carcinogens was reported to inhibit colon carcinogenesis, possibly by suppression of Phase I enzymes. Anti-metastatic effects were detected when bovine lactoferrin was given intragastrically to mice bearing highly metastatic colon carcinoma 26 cells. A marked increase in the number of cytotoxic T and NK cells in the mucosal layer of the small intestine and peripheral blood cells was thus found.

[0015] Wang W-P et al.: "Activation of Intestinal Mucosal Immunity in Tumor-bearing Mice by Lactoferrin", Japanese Journal of Cancer Research, Japanese Cancer Association, Tokyo, JP, vol.91, 1 October 2000, pages 1022-1027. This document discloses that oral administration of bovine lactoferrin is responsible for increasing CD4(+) and CD8(+) T cells and NK (asialoGM1(+)) cells in the blood of tumor-bearing mice. This document also discloses that oral administration of bovine lactoferrin and bovine lactoferrin-hydrolysate is associated with strong increases in CD4(+) and CD8(+) T, as well as asialoGM1(+) cells in lymphoid tissues and lamina propria of the small intestine in mice.

[0016] Tsuda H et al.: "Cancer prevention by bovine lactoferrin and underlying mechanisms-a review of experimental and clinical studies", Biochemistry and Cell Biology. Biochimie et Biologie Cellulaire, NRC Research Press, CA, vol. 80, 1 January 2002, pages 131-136. This document discloses that bovine lactoferrin has been found to significantly inhibit colon, oesophagus, lung and bladder carcinogenesis in rats when administered orally. Concomitant administration of bovine lactoferrin with carcinogens was reported to inhibit colon carcinogenesis, possibly by suppression of Phase I enzymes. Anti-metastatic effects were detected when bovine lactoferrin was given intragastrically to mice bearing highly metastatic colon carcinoma 26 cells. A marked increase in the number of cytotoxic T and NK cells in the mucosal layer of the small intestine and peripheral blood cells was thus found.

[0017] Kasaian M T et al.: "Cyclosporin A Inhibition of Interleukin 2 Gene Expression, but not Natural Killer Cell Proliferation, after Interferon Induction in Vivo", Journal of Experimental Medicine, Rockefeller University Press, JP, vol. 171, 1 March 1990, pages 745-762. This document discloses that NK cell cytolytic activity is enhanced after exposure to interferons and/or interferon inducers such as polyinosinic-polycytidylic acid (polyIC).

[0018] Riccardi C et al.: "Generation of Mouse Natural Killer (NK) Cell Activity: Effect of Interleukin-2 (IL-2) and Interferon (IFN) on the in vivo Development of Natural Killer Cells from Bone Marrow (BM) Progenitor Cells", International Journal of Cancer, John Wiley & Sons, Inc, vol.38, 1 January 1986, pages 553-562. This document discloses that *in vivo* administration of interferon or polyIC was able to cause an earlier maturation of NK activity after irradiation of a mouse. In contrast, a significant inhibition of NK activity was found in interferon-treated animals after irradiation and a bone marrow graft. Pre-treatment of donor bone marrow with polyIC or interferon was also able to induce a more rapid reconstitution of NK activity of recipient mice. A synergistic effect was obtained when irradiated mice were transplanted with polyIC pre-treated bone marrow and then treated with interleukin-2.

[0019] Kuhara T et al.: Bovine Lactofferin no Keiko Toyo niyoru NK Saibo Kassei no Zoka to Sono Sayo Kijo (Oral administration of lactoferrin raises NK cell activity in mice)", Miruku Saiensu - Milk Science, Hon Rakuno Kagakukai, Sendai, Miyagi-Ken, NI, vol.53, no.4, 1 December 2004, pages 262-264. This document discloses that bovine lactoferrin increases NK cell activity ofNK cells in the spleen and in peripheral blood.

[0020] US 5 914 346 A. This patent application discloses a method of enhancing the activity of NK cells by administering orally or parenterally conjugated linoleic acids, or nontoxic salts, active esters, active isomers, or active metabolites thereof and mixtures thereof.

[0021] Testi R et al.: "Sequential Metabolic Events and Morphological Changes During in vivo Large Granular Lymphocyte Activation and Proliferation", Cellular Immunology, Academic Press, San Diego, CA, US, vol. 102, no. 1, 1 October 1986, pages 78-88. This document discloses the effects of the polyIC injection on NK cell activity and large granular lymphocyte (LGL) number in the peripheral blood. It was observed that the NK cell activity and LGL number reached maximal levels by 12 hours after injection, remained on a plateau 24 to 48 hours later, then slightly decreased

on day 4 , and returned to control levels by day 6.

Disclosure of the Invention

[0022] An object of the present invention is to provide a method for screening for a substance having an action of proliferating NK cells.

[0023] When the inventors of the present invention examined a method for screening for a food or drink-derived factor that enhances the NK activity, they administered lactoferrin, which is a food (milk)-derived factor, to an animal for a predetermined period, then administered a Toll-like receptor ligand with specific timing during the administration period, and found that the proportion of NK cells among the intraperitoneal cells markedly increased in the peritoneal cavity of the animal. Further, they found that since it became possible to induce NK cells in the peritoneal cavity by the aforementioned method, the NK activity could be easily analyzed and NK cells could be conveniently collected.

[0024] The invention of the present application is a method for screening for a substance having an action of proliferating natural killer (NK) cells in a living body of an animal (except for human), which comprises: administering a test substance orally to the animal every day for 7 days, administering polyinosinic-polycytidylic acid single dose intraperitoneally to the animal at 3 days before the completion of the administration of the test substance, for enhancing the action of the test substance of proliferating NK cells, detecting induction of the proliferation of the NK cells in a peritoneal cavity in the animal, and selecting test substances capable of proliferating NK cells.

[0025] Optionally, the test substance is food, drink or a component thereof.

Best Mode for Carrying out the Invention

[0026] Hereafter, preferred embodiments of the present invention will be explained in detail. However, the present invention is not limited to the preferred embodiments described below. In the present specification, percentage is used on mass basis unless otherwise indicated.

[0027] As the Toll-like receptor ligand used for the present invention, polyinosinic-polycytidylic acid (polyIC) is used. PolyIC is particularly preferably used from a viewpoint of inducing and proliferating NK cells in an animal to which the NK cell proliferating drug of the present invention is administered, preferably in the peritoneal cavity of the animal. In an alternative embodiment that does not form part of the present invention, any Toll-like receptor ligand can be used, so long as the ligand recognizes one or more of the family members of Toll-like receptors 1 to 10 currently confirmed. In particular, lipopolysaccharides, β-glucans, double-stranded RNAs, and anticancer agents such as taxol, defensin, heat shock proteins, fibrinogen, hyaluronic acid degradation products and so forth. However, even Toll-like receptor ligands that have not been confirmed to date can also be used so long as they can proliferate animal NK cells.

[0028] A first agent (test substance) is preferably administered in an amount of 10 to 2000 mg/day, more preferably 100 to 1000 mg/day, per kg body weight of an animal in terms of the amount of the test substance. Although the method of administration is not particularly limited, oral administration is preferred. Further, the aforementioned administration amount is preferably divided and administered one or more times per day everyday for 5 to 10 days, more preferably everyday for 7 to 8 days.

[0029] A second agent (polyIC) is preferably administered in an amount of 10 to 1000 μg/day, more preferably 50 to 200 μg/day, further preferably about 100 μg/day, per kg body weight of an animal in terms of the amount of polyIC. The second agent is preferably administered as a single dose of the aforementioned amount, and a single dose of about 100 μg/kg is particularly preferably administered. Although the method of administering the second agent is not particularly limited, intraperitoneal administration is preferred. Further, the second agent is preferably administered once 5 to 2 days, preferably 3 days, before the completion of the administration of the test substance.

[0030] The dosage forms of the first agent and the second agent are not particularly limited so long as they are prepared to be suitable for the aforementioned administration method and schedule. Further, the first agent and the second agent may contain other ingredients that do not adversely affect storage or impair actions of these ingredients, such as carriers, excipients and pH modifiers, in addition to the test substance and the Toll-like receptor ligand.

[0031] The aforementioned animal is not particularly limited so long as NK cells are induced and proliferated by administration of lactoferrin as a test substance and a Toll-like receptor ligand, and examples thereof include mouse, rat, goat, sheep, horse, bovine and so forth.

[0032] The preferred timing of administration of a Toll-like receptor ligand can be determined by performing a preliminary experiment so that NK cells should be efficiently induced in an animal used. That is, a suitable condition can be determined by administering a Toll-like receptor ligand to animals to which lactoferrin as a test substance is administered everyday with changing the duration from the scheduled day of the completion of the administration of lactoferrin and comparing induction of NK cells. Once a suitable condition is determined, the administration schedule can be established according to this condition when the same type of animals are used.

[0033] Proliferation of NK cells can be confirmed by, for example, using a labeled antibody that recognizes NK cells

to measure the proportion of positive cells that react with the antibody among cells collected from an animal organ or tissue, for example, the peritoneal cavity. For example, when a mouse is used as the animal, an anti-CD49b/Pan-NK cell antibody (e.g., one produced by Pharmingen) can be used, and the proportion of cells positive for the CD49b/Pan-NK cell antibody can be measured to confirm NK cells. In this measurement, antigens of anti-mouse CD16/CD32 antibody or anti-rat CD32 antibody are preferably blocked by using these antibodies to reduce antibody binding via CD16/CD32 (FcγIII/II receptor) relating to the background. When C57BL/6 (strain) is used as a mouse, the proportion of NK1.1 positive cells is preferably measured by using the anti-NK1.1 antibody as an antibody that recognizes NK cells. Specifically, as described in the examples, cells can be stained by using FITC-labeled NK1.1 antibodies, and the proportion of NK1.1 positive cells can be measured by using a flow cytometer (e.g., FACS™ Calibur, Becton, Dickinson and Company).

[0034] By the present invention, it has been revealed that the NK cell proliferating action of lactoferrin as a test substance was enhanced by administration of polyIC. Therefore, by using polyIC, the action of a substance having an NK cell proliferating action can be enhanced, and screening can be performed for a substance having an NK cell proliferating action with high sensitivity. That is, screening can be performed for a substance having an NK cell proliferating action by administering a test substance and a Toll-like receptor ligand to an animal and detecting induction of NK cells in the animal.

[0035] Specifically, for example, when a test substance and polyIC are administered to an animal according to the schedule for administration of lactoferrin determined as described above, if NK cells are induced in the animal, the aforementioned test substance is considered to have an NK cell proliferating action.

[0036] The administration method may be similar to that described above.

[0037] Preferably, the test substance is orally administered, and polyIC is intraperitoneally administered. Further, induction of NK cells can be detected by, for example, using a labeled antibody that recognizes NK cells and measuring the proportion of positive cells that react with the antibody as described above. For the test substance for which induction of NK cells is confirmed as described above, it is preferable to further administer the test substance alone to an animal and confirm proliferation ofNK cells in an organ or tissue of the animal such as the spleen.

[0038] Examples of the preferred test substance include food and drink such as milk of animals and various components of these food and drink.

[0039] A substance having an NK cell proliferating action found by the screening method of the present invention, in particular, food or a component thereof, can be utilized as a drug, health food or a component thereof intended to be used for proliferation of NK cells or prophylactic treatment of a disease that can be prevented by proliferation of NK cells.

Examples

[0040] The present invention will be explained more specifically with reference to the following examples. However, the scope of the present invention is not limited to these examples.

Example 1

[0041] In this example, a test was performed in order to examine the NK cell proliferating effect of lactoferrin.

(1) Test method

[0042] Forty five 7-week old C57BL/6 mice (purchased from Charles River Laboratories Japan, Inc.) were divided into 5 groups, each consisting of 9 animals, acclimatized and fed for 1 week. A solution of bovine lactoferrin (Morinaga Milk Industry Co., Ltd.) in physiological saline (Otsuka Pharmaceutical Co., Ltd.) was administered everyday to the animals of each group for 7 days in an amount of 0 (control sample), 30, 100, 300 or 1000 mg/kg body weight by using an oral sonde. After completion of the administration, the animals were left for 1 day, and the spleen was collected from each mouse, and a suspension of spleen cells was prepared. The number of cells in the prepared cell suspension was adjusted to 2 x 10^6 cells per specimen. The aforementioned cell suspension was added with 10 μl per specimen of anti-mouse CD16/CD32 monoclonal antibody (Fc Block™, Becton, Dickinson and Company) solution diluted to a concentration of 1 μg/10 μl with a Cell Wash (Becton, Dickinson and Company) solution containing 1% bovine fetal serum (henceforth referred to as "dilution buffer") and left standing on ice for 5 minutes to block the FcγIII/II receptor.

[0043] Then, the mixture was added with 10 μl of FITC-labeled anti-mouse NK1.1 antibody (Pharmingen) solution adjusted to a concentration of 1 μg/10 μl with the dilution buffer, mixed and left standing for 25 minutes to stain NK1.1 positive NK cells. Subsequently, the mixture was added with 500 μl of the dilution buffer and centrifuged at 3500 rpm for 5 minutes to wash the cells, and this washing procedure was repeated twice. Then, the cells were added with 500 μl of FACS™ Lysing Solution (Becton, Dickinson and Company) and left standing at room temperature for 10 minutes to dissolve erythrocytes. Subsequently, the cells were washed twice according to the aforementioned washing procedure, and recovered cells were added with 1 ml of the dilution buffer to prepare a cell suspension. The prepared cell suspension

was filtered through a nylon screen (Flon Industry Co., Ltd., Product No.: F-3100-134) and collected in a polystyrene tube, and the proportion of NK1.1 positive cells was measured by using a flow cytometer (FACS™ Calibur, Becton, Dickinson and Company).

(2) Test results

[0044] As a result, it was found that the proportion of the NK1.1 positive cells, that is, NK cells, among the spleen cells dose-dependently increased in the administration group receiving lactoferrin in an amount of 100 to 300 mg/kg body weight.

Example 2

[0045] In this example, a test was performed in order to examine the NK cell proliferating and inducing effect of lactoferrin and a Toll-like receptor ligand.

(1) Test method

[0046] In the same manner as in Example 1, 9 mice per group, 63 mice in total, were acclimatized and fed for 1 week. Among the groups, 3 groups were assigned lactoferrin administration groups (LF groups, dose of lactoferrin: 300 mg/kg body weight/day), and the remaining 4 groups were assigned control groups (physiological saline was administered). An administration schedule was designed, in which each sample (lactoferrin or physiological saline) was administered everyday for 7 days to mice in all the 7 groups by using an oral sonde, then nothing was administered for 1 day, and the 8th day was assumed as the day of completion of the administration. Among the 3 lactoferrin administration groups and 3 groups of the control groups, groups of which mice were intraperitoneally administered with 100 μg of polyIC (Polyinosinic-Polycytidylic Acid Sodium Salt, Product No.: P-0913, Sigma) 7 days, 3 days or 1 day before completion of the administration were assigned, respectively, and the remaining one control group was assigned a group administered with no polyIC for use in the test.

[0047] After completion of the administration, 5 ml of Hank's balanced salt solution (Hank's solution "Nissui", Nissui Pharmaceutical Co., Ltd.) was injected into the peritoneal cavities of the mice in each group to wash the inside of the peritoneal cavities, this procedure was repeated twice, and the washing solution was collected to prepare intraperitoneal cell suspensions. Subsequently, in the same manner as described in Example 1, intraperitoneal cells were stained by using FITC-labeled NK1.1 antibody (Pharmingen), and the proportion of NK1.1 positive cells was measured by using a flow cytometer (FACS™ Calibur, Becton, Dickinson and Company).

(2) Test results

[0048] The results of the above test are shown in Table 1. As a result, it was found that the proportion of NK cells and the absolute number of NK cells in the peritoneal cavity markedly increased in the group administered with polyIC 3 days before completion of the administration among the lactoferrin administration groups. However, in the other lactoferrin administration groups and control groups, significant increases were not confirmed in the proportion of NK cells or the absolute number of NK cells in the peritoneal cavities. Although increases in the proportion of NK cells and the absolute number ofNK cells were confirmed in the spleens in all the 3 lactoferrin administration groups, such marked change as confirmed above in the peritoneal cavities was not confirmed.

[0049] From the above results, it was found that, under the aforementioned administration conditions, NK cells could be specifically induced in the peritoneal cavity, and the proportion and the absolute number of NK cells could be markedly increased, in particular, in the administration schedule of administering lactoferrin everyday for 7 days and completing the administration on the 8th day, by administering polyIC 3 days before completion of the administration.

Table 1

Administration of polyIC		Proportion of NK cells (%)	Number of NK cells (x 10^5)
No administration	LF group	4.68 ± 0.98	1.58 ± 0.57
	Control group	3.97 ± 1.84	1.55 ± 0.83
7 days before completion of administration	LF group	3.77 ± 1.89	1.51 ± 1.06
	Control Group	4.17 ± 1.88	1.43 ± 0.98

(continued)

Administration of polyIC		Proportion of NK cells (%)	Number of NK cells (x 10^5)
3 days before completion of administration	LF group	11.28 ± 4.53*	5.56 ± 2.45*
	Control Group	7.41 ± 2.70	1.99 ± 1.01
1 day before completion of administration	LF group	4.89 ± 1.33	1.35 ± 0.63
	Control group	3.83 ± 1.81	0.95 ± 0.87

* represents significance level $p < 0.05$ in the Tukey-Kramer's test performed with respect to the control groups.

[0050] From the results of Examples 1 and 2, it was confirmed that NK cells were proliferated at least in the spleen by oral administration of lactoferrin, and that NK cells were induced in the peritoneal cavity by further intraperitoneally administering polyIC. From the above, it is considered that if another substance having an action of proliferating NK cells in a living body of an animal is administered instead of lactoferrin, and a Toll-like receptor ligand such as polyIC is, for example, intraperitoneally administered, NK cells are also induced in the peritoneal cavity. It is considered that such induction of NK cells using a substance having an NK cell proliferating action and a Toll-like receptor ligand in combination can be utilized in screening for a substance having an NK cell proliferating action.

Industrial Applicability

[0051] Major advantages provided by the present invention are as follows.

(1) NK cells of animals including human can be specifically induced and proliferated in a large amount by administering lactoferrin and a Toll-like receptor ligand, in particular, polyIC. In this case, since NK cells are not hardly contaminated with other cell groups, improvement of the collection amount and purity of NK cells are expected in the preparation using a labeled antibody.
(2) Since NK cells can be efficiently obtained while maintaining a high cytotoxic activity, they can be utilized as a drug for cell-mediated immunity therapy such as treatment of malignant tumor or the like.
(3) Screening can be performed for a factor derived from food or drink (diet) that enhances or activates the natural killer activity (especially against virus) *in vivo* by oral administration without using radioactivity or special facilities.

Claims

1. A method for screening for a substance having an action of proliferating natural killer (NK) cells in a living body of an animal (except for human), which comprises:

   administering a test substance orally to the animal every day for 7 days,
   administering polyinosinic-polycytidylic acid single dose intraperitoneally to the animal at 3 days before the completion of the administration of the test substance, for enhancing the action of the test substance of proliferating NK cells,
   detecting induction of the proliferation of the NK cells in a peritoneal cavity in the animal, and
   selecting test substances capable of proliferating NK cells.

2. The method according to claim 1, wherein the test substance is food, drink or a component thereof.

Patentansprüche

1. Ein Verfahren zum Screenen einer Substanz, die eine proliferierende Wirkung auf natürliche Killerzellen (NK-Zellen) in einem lebenden Körper eines Tieres (ausgenommen Menschen) aufweist, das umfasst:

   orales Verabreichen einer Testsubstanz einem Tier täglich über 7 Tage,
   intraperitoneales Verabreichen einer Einzeldosis Polyinosin-Polycytidylsäure einem Tier 3 Tage vor der Beendigung der Verabreichung der Testsubstanz, um die proliferierende Wirkung der Testsubstanz aufNK-Zellen zu erhöhen,

Ermitteln der Induktion der Proliferation der NK-Zellen in einer Peritonealhöhle in dem Tier und Auswählen von Testsubstanzen, die zur Proliferation von NK-Zellen geeignet sind.

2. Das Verfahren gemäß Anspruch 1, wobei die Testsubstanz ein Nahrungsmittel, Getränk oder ein Bestandteil davon ist.

Revendications

1. Procédé de criblage d'une substance ayant une action consistant à faire proliférer des cellules tueuses NK dans le corps d'un animal vivant non humain comprenant les étapes consistant à :

administrer à l'animal par voie orale une substance test tous les jours pendant 7 jour,
administrer par voie intra-péritonéale une seule dose d'acide polyinosinique-polycytidylique à l'animal trois jours avant l'achèvement de l'administration de la substance test pour augmenter l'action de la substance test consistant à faire proliférer les cellules NK,
détecter l'induction de la prolifération des cellules NK dans la cavité péritonéale de l'animal, et
choisir des substances tests aptes à faire proliférer les cellules NK.

2. Procédé conforme à la revendication 1, selon lequel la substance teste est un aliment, une boisson ou une partie d'un aliment ou d'une boisson.

REFERENCES CITED IN THE DESCRIPTION

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